ISSN: 2395-0536 Impact Factor- 2.95*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

IN VITRO AND *IN VIVO* EVALUATION OF ANTIDIABETIC ACTIVITY OF PHYTIC ACID

J.Lalitha Rani, A.Bala Yamini, M.Abhinay, G.Nireekhna Rao, R.Dineesha, B.Bhavana, V.Vidya

Department of Pharmacology, JITS College of Pharmacy, Kalagampudi, Palakol, Andhra Pradesh,, India.

ABSTRACT

Aim of the study is to investigate phytic acid for antidiabetic activity using *in vivo* and *in vitro* models. The decreased body weight in diabetic rats is due to excessive breakdown of tissue protein. Treatment with phytic acid or glimepiride improved body weight significantly inducing prevention of muscle wasting due to hyperglycemic condition. Administration of phytic acid to diabetic rats reduced the glycosylation of haemoglobin by virtue of its normo glycemic activity and thus decreases the level of glycosylated A1c in diabetic rats.

Key Words: phytic acid, antidiabetic activity

Author for correspondence
J.Lalitha Rani,
Department of Pharmacology,
JITS College of Pharmacy, Kalagampudi,
Andhra Pradesh, India.
Email: lilatharanisattineedi@gmail.com

INTRODUCTION

Ancient Indian Sanskrit literature that deals with the health care system describes the diabetes mellitus as ‘madhumeha’. Current studies conducted estimates that diabetes mellitus is most popular and commonly appearing disorder in human population all over the world. Recent developments in modern science helps to understand the pathophysiology of diabetes mellitus and rely on the ayurvedic and modern medicine to prevent and treat this disorder. These developments help to identify the potential constituents and develop novel therapies from traditional medicinal plants to combat the diabetes with lesser side effects. Imbalance in physiological homeostasis generates several diseases and recognizes that life is based on a complex and finely tuned network of reduction-oxidation (redox) reactions that are under homeostasis factors that can alter this redox balance, often resulting in overt generation of free-radicals (oxidative stress). Oxidative stress is one of the major causative factor for development of number of diseases like diabetes mellitus, atherosclerosis, cancer, neurodegerative diseases and ageing due to imbalance of oxidant and antioxidant status in the human body. Therefore, modern science have initiated several global programmes to harness and harvest natural antioxidant rich resources and boost antioxidant defense in the human body by various means (1, 2). In order to manage carbohydrate - related metabolic disturbances at various levels, several medicines have been developed. For example, to manage post-prandial hyperglycemia (PPHG) at digestive level, modern medicine has α-glucosidase inhibitors (acarbose, miglitol and voglibose), to tackle insulin insufficiency and insulinotropic action at β-cells of pancreas, sulphonyl ureas (glypizide, glibenclamide) to enhance glucose uptake, biguanides (metformin) and to manage the problems of insulin resistance, insulin sensitizers (glitazones) are developed. Diabetes mellitus is a complex disorder that demands

multi-modal therapy. Combination of various types of herbal formulations are called polyherbal formulations. They produce the synergistic, potentiatic, agonistic/antagonistic pharmacological actions thereby dynamically producing maximum therapeutic efficacy with minimal side effects. Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. It is a chronic disorder characterized by a high blood glucose concentration which is called as hyperglycemia and caused due to insulin deficiency and insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced up take of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption exceeds, glucose spills over into urine (polyuria), which inturns, results in dehydration, thrist and increased drinking (polydipsia). Insulin deficiency causes wasting through increased breakdown and reduced synthesis of protein. Diabetic ketoacidosis is an acute emergency, that develops in the absence of insulin because of accelerated fat breakdown to acetyl-CoA, in the absence of aerobic carbohydrate metabolism which is converted to acetoacetate and β-hydroxybutyrate (which cause acidosis) and acetone (ketone) (1-3). Phytic acid (myo-inositol hexakisphosphate) is an abundant plant constituent. Edible legumes, cereals, oil seeds, pollen, nuts and citrus fruits supply a substance called phytic acid which releases inositol when acted on by bacteria in the digestive tract. Phytic acid was found to possess antioxidant, anticancer, analgesic, platelet aggregation, dyslipidemia etc. The multiple effects possessed by phytic acid made us to investigate the antidiabetic activity using *in vivo* and *in vitro* models.

MATERIALS AND METHODS

Induction of diabetes

The animals were fasted overnight and rendered diabetic by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (60 mg/kg *b.w.*) in 0.1 M citrate buffer, pH 4.5, after a baseline blood glucose estimation. The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Five days later, rats with blood glucose levels above 250 mg/dl were considered diabetic and included in the study.[67]

Study design

Normal (non-diabetic) rats were treated with saline (n = 6 per group). The diabetic rats (n = 6) were divided into 3 groups: the first group was treated with saline, the second group treated with 650 mg/kg, *b.w.* of phytic acid[68] and the third group was treated with 2.5 mg/ kg, *b.w* of glimepiride.[69] The various treatment were started 5 days after the i.p administration of STZ and considered as day 0 of diabetes. Drugs were administered orally and treatment was continued for 28 days. The doses employed for all drugs were within therapeutic range to suit the experimental animal used. i.e. the rat.[70]

Sample collection

Blood sample were collected from tip of rat tail and blood glucose levels were estimated using an electronic glucometer (Accu-Chek Active, Ireland)

Estimation

Blood samples were drawn at weekly intervals till end of study (i.e. 28 days). Fasting blood glucose estimation and body weight measurement were done on the day 0, 7, 14 and 28 of the study. Haemoglobn A1c levels were measured on zero (pre and post treatment) and 28 days of treatment. On day 28, blood was withdrawn by retro-orbital puncture from all fasted rats in each group. Serum was separated and analyzed for serum cholesterol, triglycerides, HDL, LDL and VLDL levels using standard commercial diagnostic kits (Agappe Diagnostics, Kerala) following manufacturers instruction in a semi autoanalyser (Mispa Excel Chemistry Analyzer, Mumbai) and values were tabulated. Rats were sacrificed by cervical dislocation under mild ether anaesthesia on day 28 and tissues were used for preparation of homogenate and histological studies (4-6).

Histopathological studies

The whole pancreas from each animal was removed after killing the animals, was placed in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 cm thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. The photomicrogaphs of histological studies are presented in figure (9A-9D).[72]

Statistical analysis

Values are expressed as mean ± standard error mean (SEM) and analyzed using statistical package for social sciences (SPSS) version 7.5 using ANOVA followed by Dunnett's test. P<0.01 were considered significant.

RESULTS AND DISCUSSION

The biological evaluation was carried out in 650 mg/kg dose of phytic acid for 28 days treatment. Table-1 shows the body weight of control and experimental animals on 0, 7, 14 and 28 days of treatment. Results showed no intra group variation in the basal body weight. There was significant reduction of body weight in diabetic control animals compared to phytic acid and glimepiride treated animals. The 0 day body weight showed no difference in all groups. On 7th day significant reduction of body weight in diabetic control animals when compared with control and treated animals. Control animals have shown a body weight of 250.33 g. Body weight of diabetic control rats were 202.5 g. Rats treated with phytic acid and glimepiride showed the body weight 233.6 g and 240 g respectively. Reduced body weight indicates the induction of diabetes. The normal control and drug treated rats gained significant weight (p< 0.01) on day 14 and 28. However, the increase in treated rats were significantly lower than non diabetic rats (p<0.01).

Table-1 Effect of Phytic acid on body weight

Treatment	Body weight (g)			
	0 day	**7th day**	**14th day**	**28th day**
Control	247.5±1.70	250.33±1.68	254.66±1.78	260±1.43
Diabetic control	244.16±2.007*	202.5±3.09*	192.3±3.08*	185.16±2.68*
Phytic acid	245.0±1.82*	233.6±1.94*	249.5±2.07*	260.5±2.23*
Glimepiride	245.0±1.82*	240.0±3.27*	252.83±2.33*	262.5±2.27*

Data are expressed as mean ± SEM; n=6 animals in each group. Values are statistically significant at P<0.01. Diabetic control was compared with control rats. Diabetic + Phytic acid and diabetic + glimepiride were compared with diabetic control. Table- 2 shows the level of blood glucose in normal and experimental animals on 0, 7, 14 and 28 days of drug treatment. There was a significant reduction of blood glucose in phytic acid and glimepiride treated hyperglycemic animals compared to diabetic control animals. The 0 day glucose level indicates the levels after 5 days of streptozotocin treatment. Control animals have shown a blood glucose level of 77.83 mg/dl. Blood glucose level of diabetic control rats were 277.33 mg/dl. Rats treated with phytic acid and glimepiride showed the blood glucose levels of 277 mg/dl and 278.5 mg/dl respectively. These high blood glucose levels indicates the induction of diabetes. On the 7th and 14th day, there was change in blood glucose levels in diabetic control rats, but there was a significant (p<0.01) decrease in the blood glucose level of the rats treated with phytic acid and glimepiride. Administration of phytic acid upto 28 days tends to bring the blood glucose levels towards normal. The phytic acid treated groups showed blood glucose level of 94.66 mg/dl, when compared with that of diabetic control group (411.83 mg/dl). A similar reduction in blood glucose level was noticed in glimepiride treated rats (86.33 mg/dl).

Table-2 Effect of Phytic acid on blood glucose and glycosylated haemoglobin level of experimental rats

Drug treatment	Blood glucose (mg/dl)				Glycosylated haemoglobin (%)		
					Pre treatment	Post treatment	
	0 day	7th day	14th day	28th day	0 day	0 day	28th day
Control (Normal Saline 10ml/kg)	77.83 ± 1.66	80.00 ± 1.26	79.50 ± 1.47	78.66 ± 1.28	4.72 ± 0.81	4.70 ± 0.80	4.96 ± 0.33
Diabetic control (Streptozotocin 60 mg/kg)	277.33 ±11.38*	353.66 ±12.49*	381 ±13.03*	411.83 ±10.83*	4.95 ± 0.56*	10.67 ± 0.30*	10.29 ± 0.19*
Phytic acid (650 mg/kg)	277 ±11.34*	204.66 ±4.31*	145.83 ±3.91*	94.66 ±2.57*	4.98 ± 0 19*	10.67 ±0.30*	5.27 ± 0.20*
Glimepiride (2.5 mg/kg)	278.5 ±11.02*	179 ± 5.42*	122.83 ±3.26*	86.33 ±1.64*	4.63 ± 0.28*	10.65 ±0.23*	4.80 ± 0.23*

Histopathological examination revealed extensive alterations in the pancreas of streptozotocin induced diabetic rats. The pancreas of control showed normal architecture whereas diabetic control animals showed more lesions (Fig-1). The pancreas of rats treated with phytic acid (Fig-2) showed fewer lesions with mild degeneration when compared to diabetic control rats.

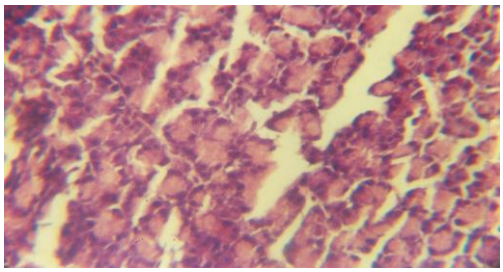

Fig-1 Section of pancreatic tissue of STZ diabetic rats showing focal interstitial pancreatitis, degeneration and severe lesions of pancreatic cells

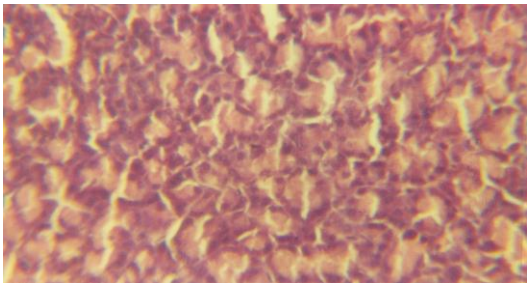

Fig-2 Section of pancreatic tissue of rats treated with phytic acid showing almost normal pancreatic histology with fewer lesions

CONCLUSION

In conclusion it may be stated that, there occurs selective decrease in the hyper glycemic state after the administration of phytic acid. Phytic acid reduces the severity of oxidative stress and acuity of hyperglycemia (induced by STZ), a process that is closely linked to glucose oxidation and formation of free radicals. The results suggested that phytic acid has more favourable effect on lipid profile in STZ-induced diabetic rats, compared with glimepiride as well as regeneration of β-cells of pancreas. The *in vitro* studies have shown an inhibitory effect on α-amylase and α-glucosidase enzymes which are involved in postprandial hyperglycemia. The present study suggest that phytic acid can be successfully utilized for the management of diabetes due to their hypoglycemic action. Further studies on the nature of functional group involved would enlighten the exact mechanism and thus help to rationalize their use in the treatment of diabetes more effectively.

REFERENCES

1.Tiwari AK. Wisdom of Ayurveda in perceiving diabetes: Enigma of therapeutic recognition. *Curr Sci* 2015;88(7):1043-51.

2.Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospectus. *Curr Sci* 2012;83(1):30-8.

3.Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. 5th ed. New Delhi: Elsevier; 2015.p.385-7

4.Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A, Subratty AH. Screening of traditional anti-diabetic medicinal plants of mauritius for possible α-amylase inhibitory effects *in vitro*. *Phytother Res* 2016; 20:228-31.

5.Kim J, Kwon C, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci Biotechnol Biochem* 2010;64(11):2458-61.

6.Chandalia HB, Krishnaswamy PR. Glycated hemoglobin. *Curr Sci* 2012;83(12):1522-32.